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Evaluation of Virological Procedures to Detect Fetal Human Cytomegalovirus Infection: Avidity of IgG Antibodies, Virus Detection in Amniotic Fluid and Maternal Serum

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Human cytomegalovirus (HCMV) is the most common cause of viral intrauterine infection and fetal damage largely due to maternal primary infection. Virological procedures which are able to detect HCMV fetal infection were evaluated. HCMV IgG antibodies were detected in 62.5% of the pregnant women and 1.47% had a primary infection. From March, 1992 to August, 1995, 29 seroconversions were observed, and in 64 other cases. HCMV IgM antibodies were detected in the first serological test. The mean IgG antibody avidity test (AI) was 31% for the 11 seroconversions tested and 74% in 32 cases where IgG and IgM HCMV antibodies were detected in the first serum. In the 29 HCMV seroconversions, 19 amniocentesis were carried out and 12 fetuses (41.4%) were infected in utero. In four amniotic fluids positive in culture and PCR, the fetus or newborns were infected and in one out of the two cordocentesis undertaken, hepatitis, anemia, and thrombocytopenia were noted. In four other cases, investigations seeking HCMV in amniotic fluid were negative whereas infants were infected at birth. Among the 64 cases with positive HCMV IgM and IgG antibodies detected in the first serological test, three fetuses were infected in utero, but no amniotic fluid was available in these cases. Amniotic fluids were studied in 39 cases, and HCMV detection by culture and PCR-hybridization was negative. HCMV DNA was detected in the maternal sera of five out of 21 pairs of seroconversions and in two cases on the first negative serum. The assay was also carried out on 50 of the 64 HCMV IgM positive sera. Two had detectable HCMV DNA.

KEY WORDS: congenital cytomegalovirus infection, IgG antibodies avidity, amniotic fluid, polymerase chain reaction

INTRODUCTION

Human cytomegalovirus (HCMV) is the most common cause of viral intrauterine infection affecting an estimated 1% of all live births in the United States [Stagno et al., 1990, 1982b]. Maternal infections are mostly asymptomatic. Although some cases of symptomatic congenital HCMV infection have been reported following nonmaternal primary infection, fetal damage is largely due to primary infection [Gibert et al., 1987; Fowler et al., 1992; Stagno et al., 1982b]. After HCMV primary infection, the rate of transmission of the virus to the fetus has been determinated to be 40% [Stagno et al., 1986]. Although only 10% of infected infants are symptomatic at birth, 20 to 30% of these die, and most of the survivors develop late complications [Van Lierde et al., 1992]. In addition, it has been reported that neurologic sequelae could appear during childhood, in 5 to 15% of congenitally infected infants without symptoms [Stagno et al., 1985; Skorc et al., 1991].

Prenatal diagnosis is therefore essential to detect fetuses at risk of having symptoms and late sequelae. It is clear that HCMV detection in amniotic fluid is one of the the best indicators of HCMV transmission to the fetus [Stagno et al., 1985; Meisel et al., 1990; Skorc et al., 1991]. The aims of the study were to investigate prospectively diagnostic methods of congenital HCMV infection by virological study of the amniotic fluid and maternal blood viremia and to evaluate the risk of fetal infection following maternal HCMV primary infection,

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and to compare conventional methods of HCMV detection for the detection of HCMV DNA by a polymerase chain reaction (PCR) and hybridization assay.

MATERIALS AND METHODS

HCMV Serology

HCMV IgG antibodies were determined by an ELISA (VIDAS CMV IgG® bioMerieux, France) and two tests were used for IgM: (VIDAS CMV IgM® bioMerieux, CAPTIA CMV® Murex, France). Serology was undertaken in pregnant women at the first consultation and each month when the serology was negative.

HCMV IgG Antibody Avidity

Sera were tested with a standard HCMV IgG test (Berhing IgG®) and by the urea denaturation procedure as descrbed by Hedman et al. [1989]. The patient's serum was added to wells coated with HCMV antigen. After 1 hour incubation, the wells were rinsed with 8 M urea and soaked in this solution for 5 min to remove low-affinity antibodies. The wells were then washed once in the standard rinse solution and the method was continued according to the routine HCMV IgG test. The absorbance readings of the standard test were compared with the absorbance readings obtained by the urea denaturation procedure, giving an avidity index (AI) which was expressed as a percentage: absorbance readings after urea washing/absorbance readings without urea washing × 100 = AI. An AI less than 30% indicates low avidity antibodies which are mainly found in a recent infection. As reported by Blackburn et al. [1991] and Keros et al. [in press], an AI above 50% indicates high avidity antibodies associated with endogenous reactivation or reinfection or past infection.

HCMV Isolation in Cell Culture

Viral isolation was attempted from amniotic fluid, and at birth on the urine or fetal tissues. Amniotic fluids were obtained 2 to 4 weeks after the onset of antibody production or the discovery of HCMV IgM antibodies. 2 ml of amniotic fluid were inoculated into MRC5 cells (bioMérieux, France) in 25 cm^2 flask and 500 µl in three wells of a 24-well microplate. Cell cultures were examined two times a week for cytopathic effect and discarded after 4 weeks. Rapid HCMV p72 detection was performed on shell vials after 48 hr by an immune peroxidase test using a mouse monoclonal antibody to HCMV early antigen (E 13, Biosys, France)

HCMV DNA Detection by a PCR-Hybridization Assay

The assay derived from a method described previously [Freymuth et al., 1994]. Nucleic acids were extracted from 100 µl of amniotic fluid, or from each IgM positive maternal serum as described by Ishigaki et al. [1991]. The primer and probe were defined within the conserved region of the fourth exon of the IE1 gene by Georgescu et al. [1992]: MG1 (5′-AGAGTCTGCTCTCCTAGTGT-3′) at position 2333–2352, and MG2 (5′-CTATCTCAGA-CACTGGCTCA-3′) at position 2602–2621 and the probe IL3, 5′-biotinylated, by Shibata [1988]: 5′-CTGGTGT-CACCCCCAGAGTCCCCTGTACCCGCGACTATCC-3′. Primers and probe were synthesized by the Unity of Organic Chemistry, Institut Pasteur, Paris. 10 µl of the extract was amplified in a 100 µl reaction volume containing 1.25 U of Taq polymerase (Hybaid), 0.2 mM each of the four deoxynucleoside triphosphates, 0.5 µM of primers MG1 and MG2, and the buffer system: 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM MgCL2, 0.01% (wt/vol) gelatin. The reaction was performed in a automated thermal cycler (Hybaid): 10 min at 94°C, before 40 incubation cycles: 1 min at 94°C, 1 min, at 55°C and 2 min at 72°C, and, at the end, 8 min at 72°C. The final PCR products were analysed by electrophoresis on agarose gel with an expected band at 289 bp, and by a DNA Enzyme Immunoassay where the amplified DNA hybridizes with a single stranded DNA probe coated on the wall of a microtiter plate by streptavidin-biotin bond and is detected by an anti DNA antibody (GEN-ETI-K® DEIA, Sorin). The optimal concentration of the probe required for the test was 1 ng/µl and 20 µl of the denatured amplicon was dispensed into wells. The sensitivity and specificity of this PCR-hybridization assay was comparable to those described previously (data not shown).

RESULTS

HCMV IgG Antibody and Seroconversion

HCMV primary infection is defined by seroconversion occuring during pregnancy. The presence of IgM and IgG antibodies at the first serological test is defined as a possible primary infection because onset may have occurred during pregnancy or perhaps earlier. In such cases, most of the pregnant women enrolled in this study had an amniocentesis in the course of their pregnancy. Congenital infection was subsequently defined either by a positive amniotic fluid culture, or a viruria at birth, or an HCMV detection following stillbirth or termination of pregnancy.

We screened for HCMV antibodies from March, 1992 to April, 1993; 2518 pregnant women: 62.5% (1574) having no HCMV IgG antibodies were nonimmune, and 36% (907) had HCMV IgG antibodies. HCMV primary infection was confirmed in 36 cases (1.47%).

IgG Antibody Avidity Test

For the IgG antibody avidity test, the positive sera of 11 out of the 29 seroconversions have been tested. The delay between the first seronegative serum and the appearance of HCMV antibodies varied from 8 to 180 days (mean 67 days). Seven of the 11 positive sera had an AI from 15.5 to 28.5% indicating the presence of low avidity IgG antibodies (5/7 fetus or newborn were infected in utero). Overall the mean AI was 31%. One serum had AI of 57.5% (intersample time period: 44 days) which corresponds to high affinity IgG antibodies (the newborn was found to be free of HCMV). Three sera had non-significative AI, respectively, 36.5%, 38.3%, and 38.5% (intersample time period: 30, 48, and 55 days), however the three newborns were infected. In 32 of the 64 cases where IgG and IgM HCMV antibodies were detected in

TABLE I. Results of HCMV Detection by Viral Isolation Technique and PCR-Hybridization Assay in Amniotic Fluids and Newborns From 29 HCMV Maternal Seroconversions*

	Amniotic fluid		Newborn		
			infected	not infected	NA
CULTURE and PCR	+	4	4	0	0
	−	15	4[a]	10	1[b]
	NA	10	4	5	1[c]

*NA: Not available.
[a]One amniotic fluid obtained at 10 days, 3, 6, 8 weeks after maternal seroconversion.
[b]One intrauterine death.
[c]One infant to born.

the first serum, 29 had high affinity IgG antibodies, with an AI varying from 47 to 100% (mean 74%). Two sera had a non-significant AI (39.5%, 40.5%). No newborn was infected at birth in this group. Only one serum had low avidity antibodies (AI: 24%) which could indicate a closely time-related primary infection. However the newborn was found to be free of infection.

Fetal Infection in HCMV Seroconversions

Twenty nine seroconversions were investigated from March, 1992 to August, 1995. Twelve fetuses (41.4%) were infected in utero as proved by a positive viruria at birth. Amniotic fluids were obtained in 19 cases (Table I). The amniotic fluid culture results were stratified by the day of gestation and the interval from seroconversion (Table II). The average for estimated seroconversion was 15.7 weeks of gestation for infected fetus (eight cases) and 24 weeks of gestation for non-infected newborns (11 cases) showing a significant difference. HCMV detection by viral isolation or PCR was negative in 15 amniotic fluids. But in four of these 15 cases, fetuses were infected in utero, emphasizing the possibility of false-negative amniocentesis results. The delay between maternal seroconversion and amniocentesis was respectively 11 days, 3, 6, and 8 weeks for these "false negative" cases. Four amniotic fluids were positive and HCMV detected by viral isolation and PCR (both positive in the four cases). The delay between maternal seroconversion and amniocentesis was 3, 6, 7, 8 weeks respectively in these positive cases. Termination of pregnancy was done in two cases, and HCMV was isolated in all fetal tissues: brain, liver, lung (Table III). In the two other cases of congenital HCMV infections, no clinical manifestation was observed in the newborns. Amniotic fluids were not available for various reasons: gestational age, premature delivery in 10 cases and congenital HCMV infection was observed in four cases. Cordocentesis was carried out in two pregnancies (Table III). Attempts to isolate HCMV in blood leucocytes or to detect HCMV IgM antibodies were negative, even in case no. 2 where most of the fetal tissues were infected. Nevertheless hepatitis, an anemia, and thrombocytopenia were detected in this case, confirming an acute diffuse infection of the fetus.

Positive Anti-HCMV IgM in the First Serological Test

The second part of the study concerns 64 cases where positive HCMV IgM antibodies were detected in the first serological test available during pregnancy (Table IV). Forty amniocentesis were carried out, all were negative by culture and PCR and no infant was infected at birth. In the 24 remaining cases when amniotic fluids were not available, HCMV viruria was positive at birth in three cases. In that group of pregnant women, congenital HCMV infection was observed in three out of the 64 cases (4.5%).

HCMV DNA in Maternal Sera by PCR Assay

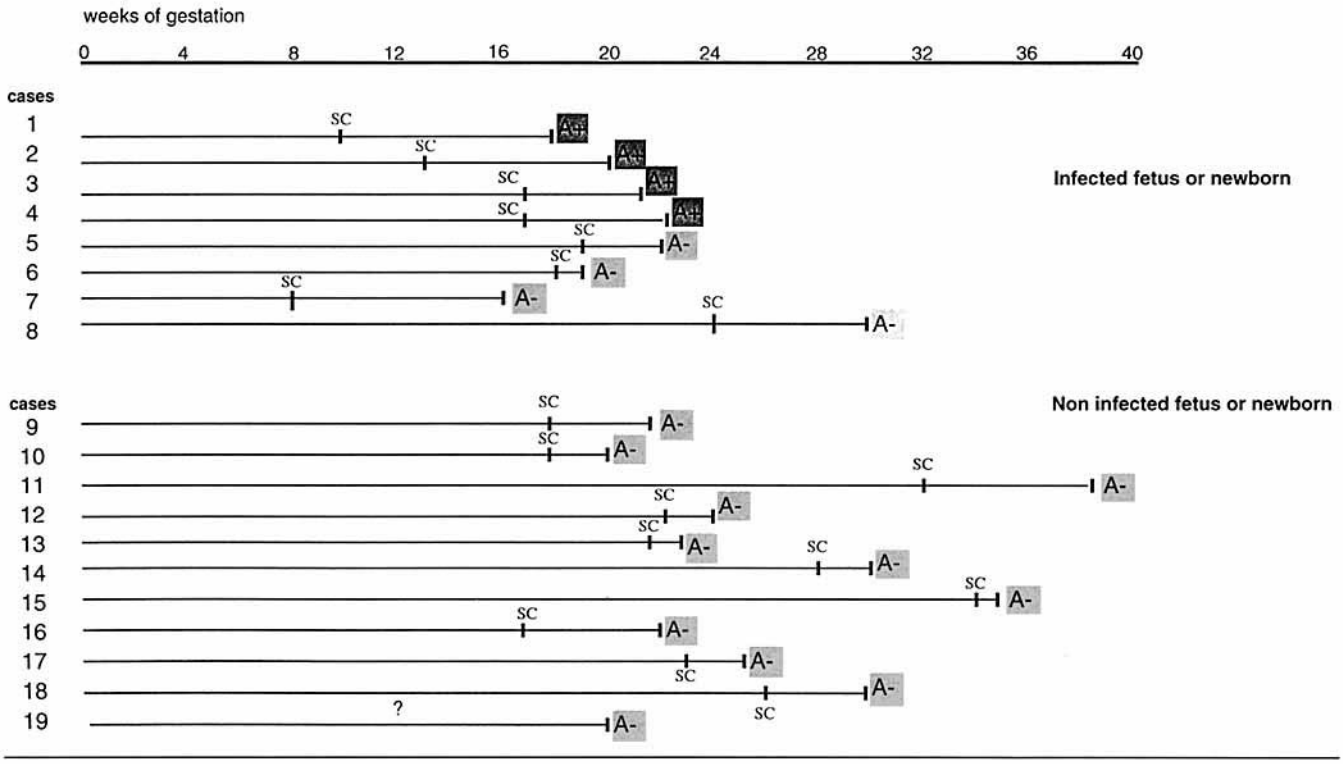
A PCR hybridization assay was carried out on 92 sera: 21 pairs of sera (Table V) with an HCMV seroconversion and 50 isolated sera with positive HCMV IgM antibodies. HCMV DNA was detected in five of the 21 seroconversions: in two cases before the onset of HCMV antibodies and in three cases together with it. The delay between the first and the second serum was 26 to 60 days. Two out of the 50 HCMV IgM positive sera had detectable HCMV DNA.

DISCUSSION

Sixty four percent of pregnant women had no HCMV IgG antibodies. The low immunity level of this population puts them at particular risk of primary infection during pregnancy. The prevalence of HCMV antibody in pregnant women has been reported to vary from 50 to 90% according to socio-economic status and geographic area of their origin. [Stagno et al., 1977; Stagno et al., 1982a; Stagno et al., 1986]. Serological diagnosis of maternal HCMV primary infection is easy in cases of seroconversion. But the discovery of HCMV IgM antibodies in a first serum obtained during pregnancy does not allow the diagnosis of an actual infection. In fact, the presence of HCMV antibodies IgM can be also the proof of secondary infection, or of persisting antibodies several months after primary infection. HCMV IgG antibody avidity could be used to differentiate primary infection from a secondary infection. The technique of denaturation by urea, has been described for rubella IgG antibodies [Hedman et al., 1989; Thomas et al., 1988]. Blackburn et al. [1991] have shown that this technique could have an extended use to differentiate primary infection from reactivation in most cases of HCMV infections. This denaturation technique is particularly useful in positive HCMV IgM antibodies sera. In our study, only one of 32 sera with positive HCMV IgM antibodies in the first serological assay and tested with IgG antibody avidity test, had low-avidity antibodies (AI = 24%), indicating a recent infection. In the remaining cases, high IgG avidity antibodies (45 to 100%) were found. This suggests that HCMV primary infection had occurred at least 3 months earlier. [Blackburn et al., 1991; Keros-Grangeot et al., in press].

In cases of documented seroconversions, we observed that HCMV foetal transmission occurred in 12 out of 29

TABLE II. Amniotic Fluid Culture Results in Relation to Weeks Interval From Seroconversion and Weeks of Gestation*



*SC: estimated seroconversion; A+: positive AF culture; A−: negative AF culture.

TABLE III. Features of Fetal Infection in Four Cases With HCMV Positive Amniotic Fluids*

	Case No. 1	Case No. 2	Case No. 3	Case No. 4
Cordocentesis				
leucocytes culture	NA	−	NA	−
elevated hepatitis enzymes	NA	+	NA	−
anemia/thrombopenia	NA	+	NA	−
HCMV IgM antibodies	NA	+	NA	−
Termination of pregnancy	yes	yes	no	no
HCMV isolation and PCR from fetal tissues or newborn				
brain	+	+	NA	NA
liver	+	+	NA	NA
lung	+	+	NA	NA
placenta	+	+	NA	NA
urine	NA	NA	+	+
pharynx	NA	NA	+	+

*NA: Not available.

studied cases (41.4%), which was consistent with current literature findings [Stagno et al., 1986]. Moreover, our results suggested that seroconversion occuring early during gestation (mean:15.7 weeks) increase vertical transmission. In the group of delivered women having positive HCMV IgM antibodies in the first serological test (64 cases), three newborns were infected (4.5%). This striking difference confirmed our belief that in this group an unknown number of primary infection took place before pregnancy.

TABLE IV. Results of HCMV Detection by Viral Isolation Technique and PCR-Hybridization in Amniotic Fluids and Newborns From 64 Positive Anti-HCMV IgM in the First Serological Test*

	Amniotic fluid		Newborn		
			infected	not infected	NA
Culture and PCR (both methods for every sample)	+	0	0	0	0
	−	40	0	29	11[a]
	NA	24	3	15	6[b]

*NA: not available.
[a]Six infants to born; four infants lost; one intrauterine death.
[b]Three infants lost; two early miscarriages; one termination of pregnancy for psychiatric indication.

TABLE V. HCMV DNA Detection in Maternal Sera by PCR-Hybridization Assay

	Pair of serum with seroconversion		Isolated serum with positive IgM
	first serum (n = 21)	second serum[a] (n = 21)	(n = 50)
HCMV IgM	0	21	50
HCMV IgG	0	20	50
HCMV DNA	2	3[b]	2

[a]From 26 to 60 days between the two sera.
[b]No pregnant women was PCR positive on the first sample.

In amniotic fluid analysis, no discordant results were noted between HCMV isolation in cell culture and HCMV DNA detection by PCR, as reported by Lynch et al. [1991]. The PCR-hybridization assay did not appear to detect HCMV earlier after infection than cell culture. But the low number of cases does not allow as a statical comparison. Usually, amniotic fluid collection was undertaken approximately 4 weeks after the first detection of HCMV IgM antibodies in maternal sera. On the other hand, Donner et al. [1994], carried out amniocentesis in 10 pregnancies following the first evidence of the presence of HCMV IgM antibodies. Viral detection was made by viral isolation and/or PCR. Five out of 10 amniotic fluids were negative on the first amniocentesis, and among the five positive fluids, three were HCMV-PCR positive although the virus did not grow in culture. On a second amniocentesis carried out later, six cases (three negative cases and three with only PCR positive on first amniocentesis) were culture positive and two were only PCR positive. It was concluded that their PCR assay is more sensitive (45.4%) than viral isolation (18.2%), allowing an earlier diagnosis. We could not confirm these results, but the primers and probe that we used in the PCR-hybridisation assay are different, even if they were located in the same exon of the IE 1 gene.

In our study HCMV was detected in amniotic fluid four times, and the viral detection after termination of pregnancy showed involvement of various organs (brain, liver, kidneys, lungs) in two cases. HCMV was isolated in the urine culture of the two remaining newborns. Isolation of HCMV in amniotic fluid has been shown to be the most reliable index for congenital infection [Lynch et al., 1991; Hohfeld et al., 1991; Apperley et al., 1988; Skvorc et al., 1991]. In the review of Grose et al. [1992] amniotic fluids were positive in all infected fetuses. Lynch et al. [1991] reported seven cases of primary maternal infection: one newborn was infected and the amniotic fluid was positive. Lamy et al. [1992] evaluated eight cases of maternal primary infection: five fetuses were infected and in all five pregnancies HCMV was detected in amniotic fluid. The largest prospective study of Hohlfeld et al. [1991] evaluated 15 maternal primary infection, with eight infected fetuses and eight positive AFs. These data show a strong correlation between fetal infection and the detection of HCMV in the amniotic fluid.

Four cases are now reported among 29 seroconversions (14%) where amniotic fluid was culture and PCR negative, in which the fetus subsequently proved to be infected. One potential possibility is that the fetus acquired infection by way of amniocentesis. We cannot exclude with certainty this possibility. The detection of a maternal viremia could be useful to assess this risk. The potential risk of introducing HCMV in the fetus by the way of amniocentesis being most likely negligible if maternal viremia was negative. Moreover, the rate of fetal transmission during maternal seroconversion in our series: 41.4%, is similar to that reported in the literature [Stagno et al., 1986; Fowler et al., 1992] suggesting that amniocentesis probably does not increase vertical transmission.

One other possible explanation could be that the samples were obtained too early, before the infection of fetus, or a sufficient viral growth in fetal tissues. HCMV is a relatively slow-growing virus (in vitro) and Apperley et al. [1988] showed that viremia is not detected before 2 or 3 weeks after primoinfection. 2 to 3 weeks would be necessary before the onset of fetal contamination and additional 2 to 3 weeks (or more) for HCMV excretion by the fetal kidneys in amniotic fluid. Thus it appears that in most of the cases, 4 to 6 weeks would be necessary for an accurate detection of potential HCMV in amniotic fluid. In our study, the delays between maternal seroconversion and amniocentesis were not significantly different between the positive cases and the false-negative cases. Some authors [Donner et al., 1994; Nicolini et al., 1994] have reported prolonged delays, from 3 to 8 weeks, in "false negative" cases while it was from 5 to 18 weeks for the positives cases.

Another possibility to explain false negative results in that both culture and PCR could be falsely negative. The presence of inhibitors of viral growth in specimens, or a low viral load could explain some of the negative results obtained in culture. For the PCR assay the use of only one pair of primers could lead to false negative results due to the variability of HCMV strains [Chou et al., 1992; Catanzarite et al., 1993]. It has been reported that the results provided by PCR remain substantially correlated with the choice of the amplified sequence. Daiminger et al. [1994] showed that the sensitivity of PCR compared to virus isolation was 67.8% for IE 1-gene primers and 94.1% for LA-gene primers.

Finally, one other possible cause of false negative results is that the antenatal diagnostic procedure was carried out too early during pregnancy. Donner et al. [1993, 1994] and Catanzarite et al. [1993] have reported false-negative cases with amniocentesis before 22 weeks of gestation. This study supports the possibility that before this time, the quantity of virus in amniotic fluid is two low and the detection of virus, even by PCR, is difficult. HCMV is excreted with the fetal urine into the amniotic fluid. Low titers of virus during the first 22 weeks of the gestation could appear if the amniotic fluid contains a very low quantity of fetal urine [Codaccioni et al., 1985]. From 20 or 22 weeks, the fetal diuresis reaches 600 to 800 ml a day and more viral particles can be found in amniotic fluid. Three of our four false negative results were observed from amniotic fluids taken at early stage of gestation: 16, 19, and 22 weeks. Some authors suggest, if amniocentesis is negative before 22 weeks of gestation, repetition of amniocentesis 4–6 week later [Donner et al., 1993, 1994; Catanzarite et al., 1993]. It seems better to us to delay amniocentesis to at least 22 weeks of gestation. Delaying amniocentesis could conflict with the ordinary criteria for considering termination of pregnancy, even if it is permitted by law.

The detection of HCMV in amniotic fluid is evidence of fetal infection, but does not seem to have a prognostic value in relation to the development of serious disease or severe sequelae. Pass et al. [1992] showed that the majority of infected infants are asymptomatic at birth

(90%), and do not develop neuropsychological or auditary sequelae. Data from most reports on the prenatal diagnosis of congenital HCMV infection by amniocentesis did not predict reliably fetal symptomatic outcome. Hohfeld et al. [1991] suggested that the study of fetal blood samples may indicate which infected fetuses have organ involvement. Elevated liver enzymes together with anemia and thrombocytopenia would indicate high risk of serious sequelae for the fetus [Hohfeld et al., 1991; Suresh et al., 1992; Nicolini et al., 1994]. Nicolini et al. [1994] reported four cases with termination of pregnancy or intrauterine death following congenital HCMV infection. HCMV was isolated from various organs in only two fetuses but disseminated infection was histologically detected in all four.

The detection of the virus or the presence of IgM in fetal blood are evidence of fetal infection but has no more prognostic value than viral detection in amniotic fluid. In our series, two infected fetus have fetal blood sampling. Specific IgM and fetal blood leucocytes culture were negative even in the fetus presenting anemia, thrombocytopenia associated with abnormal liver functions tests. Isolation of HCMV in cell culture from the fetal blood leukocytes seems to be rare, that may reflect a short duration of fetal viremia, and that the presence of specific IgM is inconsistent. The production of specific IgM appears to depend on the fetal immune system development and on the nature of infecting agent. It has been found that the majority of fetuses with congenital *rubella infection* produce specific IgM at 22 weeks [Daffos et al., 1984] whereas only 15% of fetuses infected with toxoplasma tested between 24 to 29 weeks produce specific IgM [Daffos et al., 1988]. In our study, the negativity of HCMV IgM detection may be secondary to a cordocentesis carried out at 21 and 23 weeks of gestation. Donner et al. [1993] have reported 13 HCMV congenital infections from 12 termination of pregnancies where all fetal tissue cultures were positive for HCMV. The fetal blood culture was negative in all cases, whereas HCMV IgM antibodies were detected in 9/13 cases (69%). In other data [Hohfeld et al., 1991], no cultures exhibited the presence of virus in fetal blood (0/8) whereas the sensitivity of HCMV IgM antibody detection in fetal blood varies from 60% [Nicolini et al., 1994] to 75% [Hohfeld et al., 1991].

Maternal viremia plays a key role in dissemination of HCMV infection and contamination of the placenta and the fetus. However, few results of HCMV viremia have been reported during pregnancy. In a prospective study of 92 pregnancies, Balcarek et al. [1993] have investigated the maternal viremia by peripheral blood leukocytes culture and HCMV DNA detection by PCR. Maternal viremia was detected in 33% of primary infections. Only two out of eight women had HCMV isolated from leucocytes by tissue culture technique, and seven out of eight patients had HCMV-DNA detected in leucocytes using PCR. According to Shibata et al. [1988] the PCR assay offers a much more sensitive approach to detection of HCMV viremia. Moreover, they have shown a correlation between a positive maternal viremia and the gestational age: no viremia was detected in first trimester samples and 10% (4/38) and 8% (4/50) were detected during the second and third trimester.

In our study, HCMV-DNA was found in five out of 21 sera of seroconversions (23.8%) and in two out of 50 sera (4%) from the pregnant women having positive a IgM in the first serological assay. Moreover four out of seven samples with positive serum viremia where retrieved in the third trimester, two in the second and one in the first. The results confirmed the observation of Balcarek et al. [1993] that maternal viremia is most frequently detected in case of primary infection and correlated with the increasing gestational age. There is no explanation on the easier detection of viremia in late pregnancy otherwise an increasing classical relative immunosuppression. Our results did not show that the risk of congenital infection was increased in the case of maternal viremia compared with those without evidence of viremia and others studies are necessary to know if the maternal viremia can be used as an indicator of risk of fetal infection.

Spector et al. [1992] suggested that HCMV dissemination may occur through mechanisms other than cell-to-cell transmission and to overcome the detection of latent HCMV DNA in leukocytes, the research of HCMV DNA by PCR was carried out on serum in our study. This PCR-hybridization assay is different in part from the nested-PCR described previously [Freymuth et al., 1994]. The hybridization step was chosen to overcome false positive reactions that can appear with a nested-PCR; It does not cross-react with human cellular DNA or the DNA of other Herpesviridae (HSV, VZV, CMV, EBV, HHV6, HHV7) and its detection sensitivity is 1,4 UFP [Freymuth, personal communication].

In conclusion, except for seroconversion, it is difficult to date a maternal HCMV primary infection using only HCMV IgM and IgG detection in the first serum available. The avidity index of IgG antibodies detected by urea denaturation testing could be a reasonable method for assessing recent HCMV infection. HCMV DNA detection in maternal sera should be of interest to evaluate the role of factors such as the timing of maternal viremia, the viral load and the length of in utero exposure. HCMV detection and quantification in amniotic fluid should be a specific and informative way to forecast a fetal infection. To avoid the risk of false negative results, a delay between the serological diagnosis of maternal infection and amniocentesis should be at least 4 weeks, and amniocentesis could be repeated after an initially negative result before 22 weeks of gestation. The detection of virus in amniotic fluid does not predict fetal outcome accurately. A collaborative French study is on going to evaluate pronostic markers for the risk of HCMV disease and visceral infection in fetus. Intrauterine treatment with ganciclovir or alternative methods of therapy would be considered if it was demonstrated to be safe and effective.

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